REMARKS/ARGUMENTS

The foregoing amendments in the specification and claims are of a formal nature, and do not add new matter.

Prior to the present amendment, Claims 28-47 were pending in this application and were rejected on various grounds. With this amendment, Claims 36-37 and 41-43 have been canceled without prejudice, Claims 28-35, 38-39 and 44 have been amended, and new Claims 48-57 have been added.

Claims 28-35, 38-40 and 44-57 are pending after entry of the instant amendment. Applicants expressly reserve the right to pursue any canceled matter in subsequent continuation, divisional or continuation-in-part applications.

The amendments to the specification and claims are fully supported by the specification and claims as originally filed and do not constitute new matter. In addition, new Claims 48-57 are fully supported by the specification as originally filed. Amendments to Claims 28-32 can be found in Example 150 at least on page 512, line 12 of the specification. Support new Claims 48-52 can be found at least in Example 141, starting on page 492, line 32 of the specification. Support for new Claims 53-57 can be found at least in Example 144, starting on page 508, line 30 of the specification.

1. Formal Matters

Applicants thank the Examiner for entering the Preliminary Amendment filed on December 7, 2001 into the record.

2. Priority

The Examiner states that because the present application lacks utility under 35 U.S.C. §101, Applicants are only entitled to the filing date of the present application, namely, December 7, 2001.

As discussed below, Applicants rely on the stimulatory activity in mixed lymphocyte reaction (MLR) Assay (Example 144) for patentable utility which was first disclosed in U.S.

Provisional Application No. 60/144,758 filed July 20, 1999, priority to which has been claimed in this application. Accordingly, the present application is entitled to at least the July 20, 1999 priority for subject matter defined in Claims 33-48 and 53-57. In support, Applicants enclose herewith pages 103-104, describing the MLR assay (Example 2), of the U.S. Provisional Application No. 60/144,758.

Applicants further rely on the skin vascular permeability assay (Example 141) and the chondrocyte re-differentiation assay (Example 150) for patentable utility which was first disclosed in PCT/US00/04342 filed on February 18, 2000, priority to which has been claimed in this application. Accordingly, the present application is entitled to at least the February 18, 2000 priority for subject matter defined in claims 28-52. In support, Applicants enclose herewith page 504, describing the skin vascular permeability assay (Example 141), and page 523, describing the chondrocyte re-differentiation assay (Example 150), of the PCT publication, WO 00/78961, corresponding to PCT application, PCT/US00/04342.

3. Information Disclosure Statement

In response to the Examiner's assertion that references 1 in the Information Disclosure Statement filed on September 11, 2002 and reference 2 in the Information Disclosure Statement filed on November 7, 2002 are not in proper format, Applicants file herewith, an Information Disclosure Statement listing each reference of the "Blast Search" separately and including authors/inventors, relevant accession numbers and publication dates. Applicants respectfully request that the listed information be considered by the Examiner and be made of record in the above-identified application.

4. Specification

As requested by the Examiner, the specification has been amended to remove embedded hyperlink and/or other form of browser-executable code.

The title of the application has been amended to recite a new, descriptive title indicative of the invention to which the claims are directed.

5. **Claim Objections**

Claims 28-47 were objected to for reciting a Figure number and a SEQ ID NO. Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the objection to these claims moot. Further, Applicants submit that Claims 28-35 and 38-39 have been amended to only recite SEQ ID NO. Accordingly, Applicants respectfully request that the Examiner withdraw the objection to Claims 28-35, 38-40 and 44-47.

6. **Claim Rejections Under 35 U.S.C. §101**

Claims 28-47 were rejected under 35 U.S.C. §101 allegedly "because the claimed invention is not supported by either a specific, substantial and credible asserted utility or a well established utility." The Examiner further notes that "since the protein of the invention is not supported by specific and substantial asserted utility or a well established utility, the encoding polynucleotides, vectors, host cells and methods of making proteins also lack utility." For the reasons outlined below, Applicants respectfully disagree.

Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the objection to these claims moot..

Utility – Legal Standard

According to the Utility Examination Guidelines ("Utility Guidelines"), 66 Fed. Reg. 1092 (2001) an invention complies with the utility requirement of 35 U.S.C. §101, if it has at least one asserted "specific, substantial, and credible utility" or a "well-established utility."

Under the Utility Guidelines, a utility is "specific" when it is particular to the subject matter claimed. For example, it is generally not enough to state that a nucleic acid is useful as a diagnostic without also identifying the conditions that is to be diagnosed.

The requirement of "substantial utility" defines a "real world" use, and derives from the Supreme Court's holding in *Brenner v. Manson*, 383 U.S. 519, 534 (1966) stating that "[t]he basic quid pro quo contemplated by the Constitution and the Congress for granting a patent monopoly is the benefit derived by the public from an invention with substantial utility." In

explaining the "substantial utility" standard, M.P.E.P. §2107.01 cautions, however, that Office personnel must be careful not to interpret the phrase "immediate benefit to the public" or similar formulations used in certain court decisions to mean that products or services based on the claimed invention must be "currently available" to the public in order to satisfy the utility requirement. "Rather, **any reasonable use that an applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient**, at least with regard to defining a "'substantial'" utility."' M.P.E.P. §2107.01, emphasis added. Indeed, the Guidelines for Examination of Applications for Compliance With the Utility Requirement, set forth in M.P.E.P, §2107 II(B)(1) gives the following instruction to patent examiners: "If the applicant has asserted that the claimed invention is useful for any particular practical purpose . . . and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility."

Finally, the Utility Guidelines restate the Patent Office's long established position that any asserted utility has to be "credible." "Credibility is assessed from the perspective of one of ordinary skill in the art in view of the disclosure and any other evidence of record . . . that is probative of the applicant's assertions." M.P.E.P. §2107 II(B)(1)(ii). Such a standard is presumptively satisfied unless the logic underlying the assertion is seriously flawed, or if the facts upon which the assertion is based are inconsistent with the logic underlying the assertion. Revised Interim Utility Guidelines Training Materials, 1999.

Proper Application of the Legal Standard

As discussed above, Applicants rely on the chondrocyte re-differentiation assay (Example 150, page 512, line 12 onwards) for priority and to establish patentable utility for polypeptide PRO1343. This data was first disclosed in International Application No. PCT/US00/04342 filed on February 18, 2000, the priority of which is claimed in the present application. Hence, the effective filing date of the present application is February 22, 2000 for subject matter of the instant claims directed to the chondrocyte re-differentiation activity.

Claims 28-32 as presently amended recite polypeptides that induce chondrocyte re-differentiation. Accordingly, the present application is entitled to at least the February 18, 2000 priority for Claims 28-32 (and, as a consequence, those claims dependent from the same).

It was well known at the effective filing date of the present application that chondrocytes play a key role in the synthesis and maintenance of the articular cartilage, which in turn is essential to normal joint function. Unfortunately, compared to many other tissues, articular cartilage essentially lacks the ability to regenerate following injury. One way of achieving cartilage repair, for example in osteoarthritis, is to harvest human articular chrondrocytes (HACs) from non-affected, healthy areas of the joint to be repaired. The HACs are subsequently grown in monolayer cell culture in order to produce sufficient amount of cells to fill the articular defect. Chondrocytes found in healthy joints have a round shape, and express high levels of extracellular matrix molecules, such as aggrecan, type II collagen, and link protein. In contrast, monolayer cultures of chondrocytes produce dedifferentiated fibroblast-like structures, similar to those found in the cartilage of aging and arthritic joints. (See, *e.g.,* Zhang et al., *Experimental Cell Research* 263:33-42 (2001) – copy enclosed). Accordingly, agents that are capable of inducing chondrocyte redifferentiation, as evidenced by proper differentiation of chrondrocytes in monolayer cell cultures, can be used in the treatment of joint diseases using a tissue engineering approach (See, *e.g.,* Schnabel et al., Osteoarthritis and Cartilage, 10(1):62-70 (2002) – copy enclosed). In addition, molecules capable of chondrocyte re-differentiation are promising drug candidates to repair aging or arthritic joints, for example, in which the chondrocytes have been dedifferentiated.

Hence, the polynucleotides encoding PRO1343 polypeptides meets the requirements of 35 U.S.C. §101 as being useful.

As discussed above, Applicants further rely on the skin vascular permeability assay (Assay 64 or Example 141, page 492, line 32 onwards) for priority and to establish patentable utility for polypeptide PRO1343. These results were first disclosed in International Application No. PCT/US00/04342 filed on February 18, 2000, priority to which has been claimed in this

application. Accordingly, the present application is entitled to the effective filing date of February 18, 2000 for subject matter directed to the skin vascular permeability activity.

Applicants respectfully submit that the invention defined by the presently added Claims 48-52 (and, as a consequence, those claims dependent from the same), which recite polypeptides that induce an inflammatory response, has a specific, substantial and credible asserted utility and the Claims are entitled to the effective filing date of February 18, 2000.

Example 141 describes a dye-based proinflammatory cell infiltration assay in skin in which PRO1343 induces mononuclear cell, eosinophil and PMN infiltration into the site of injection of this peptide/protein into an animal. Here, purified or conditioned media containing PRO1343 was injected intradermally onto the backs of hairless guinea pigs whereas the Evans blue dye was injected intracardially. Blemishes at the injection sites were measured 1 h and 6 h post injection. Animals were sacrificed at 6 h after injection, the skin at each injection site was biopsied, fixed in formalin and evaluated histopathologically for inflammatory cell infiltration into the skin. Such inflammatory cell infiltration assays are routinely used in the art to evaluate proinflammatory properties of novel compounds (see Rampart et al., *American Journal of Pathology* 135:21-25 (1989) – copy enclosed). For example, in Rampart et al. (see Methods, page 22), IL-8 (Interleukin 8) was identified using a similar neutrophil accumulation assay in rabbit skin and the findings were correlated with albumin flux and neutrophil dependent edema in skin.

Under proinflammatory conditions, several mechanisms act synergistically to mediate an increase in neutrophil accumulation, plasma extravasation, etc. Such events occur for example, during the acute phase of an inflammatory response to a microbial stimulus or during pathologic conditions like graft rejection, edema, psoriasis, arthritis, tissue injury etc. The enclosed reference, Rampart et al. suggests the involvement of endogenous IL-8 in an acute phase inflammatory response of an animal to a microbial stimulus and further disclosed suggestive data supporting its involvement in psoriasis (see page 24, column 1, last paragraph). Subsequent data affirmed the involvement of IL-8 in several inflammatory conditions and in immune response; for example: IL-8 has been shown to be part of the cytokine cascade in the synovium of patients

suffering from rheumatoid arthritis. IL-8 is also associated with other inflammatory diseases like asthma, leprosy, psoriasis, inflammatory bowel disease, atherosclerosis, cystic fibrosis, and in various respiratory syndromes. Similarly, a variety of real-life utilities are envisioned for PRO1343 based on the proinflammatory cell infiltration assay results disclosed herein. Thus, Applicants respectfully submit that the results from this assay have been used to identify molecules useful in treating inflammatory diseases and that PRO1343's utility lies in its use as a target for the development of anti-inflammatory agents (as is routinely done with other inflammatory molecules like prostaglandins, endothelins). Accordingly, the polynucleotides encoding PRO1343 polypeptides meets the requirements of 35 U.S.C. §101 as being useful.

Finally, Applicants rely on the data generated in the MLR assay (Example 144), first disclosed in U. S. Application Serial No. 60/144,758 filed July 20, 1999, the priority of which is claimed in the present application. Hence, the effective filing date of the present application is July 20, 1999 for subject matter directed to the T-cell proliferation activity.

Applicants respectfully submit that the invention defined by the presently added Claims 53-57 (and, as a consequence, those claims dependent from the same), which recite polypeptides that stimulates proliferation of T-lymphocytes, has a specific, substantial and credible asserted utility and the claims are entitled to the effective filing date of July 20, 1999.

Applicants submit that the presently PRO1343 polypeptide, to which the presently claimed antibodies are raised, stimulates T-cell proliferation in the MLR assay and this is described in Example 144, pages 508-509 of the specification. MLR is a well-established assay for evaluating test compounds, like polypeptide PRO1343 for their ability to stimulate T-lymphocyte proliferation *in vitro*, and consequently, for assessing the immune response of an individual. The MLR assay is well-described in standard textbooks, including, for example, *Current Protocols in Immunology*, unit 3.12; edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Institutes of Health, Published by John Wiley & Sons, Inc., which is referenced in Example 144, and, the entire content of which is expressly incorporated by reference into the disclosure of the present application. In brief, in this method, an immune response results upon mixing T-cells from antigenically distinct individuals under

cell culture conditions. An MLR reaction can be monitored qualitatively, for example, by following the incorporation of tritiated thymidine during DNA synthesis, or, by observing blast formation, or by other methods well known in the art.

Applicants further submit that the MLR assay has been extensively used and is the best *in vitro* model for screening immunosuppressive agents for use in the prevention of graft-versus-host disease and graft rejection. It is well known that the transplantation of tissues or organs between individuals with MHC incompatibilities quickly activates the recipient's immune system which then attempts to destroy the transplanted tissue or organ. Transplantation across minor histocompatibility loci generally induces a more indolent response. Physicians analyze the major and minor histocompatibility differences to predict the success of the graft and to adjust the aggressiveness of immunosuppressive therapy.

Inhibitors of MLR find utility in suppressing unwanted immune response, and thus suppresses unwanted graft rejection. For example, the ability of tepoxalin, an immunomodulatory compound, to suppress graft-versus-host reaction, has been demonstrated using the MLR assay (Fung-Leung *et al.*, *Transplantation* 60:362-8 (1995) - copy enclosed). Other immunosupressants have also been routinely identified using the MLR assay. For example, the immunosuppressive efficacy of SNF4435 and D, produced by a strain of Streptomyces spectabilis, has been tested using the MLR assay. As recently as 2002, the immunosuppressive effect of tautomycetin (TMC) was assessed with mixed lymphocyte reactions, and confirmed *in vivo* using TMC-treated rats that received a heterotopic cardiac allograft (Shim *et al., Proc. Natl. Acad. Sci USA* 99(16):10617-10622 (2002) - copy enclosed). The authors were confident to conclude from the MLR data that "TMC has the capacity to inhibit the intracellular signaling pathway leading to T cell activation and proliferation[.]" (See page 10621, second column).

Thus, the art as a whole clearly establishes that the mixed lymphocyte reaction (MLR) is a widely used *in vitro* assay for identifying immunomodulator compounds.

Applicants further submit a declaration and supportive references from the art to support the immunostimulant activity of PRO1343.

Applicants submit a declaration by Sherman Fong, Ph.D. of Genentech, Inc., an expert in the field of Immunology and co-inventor of the present application, to show that there are specific immune stimulant utilities for compounds identified by an MLR assay. The Declaration explains how the MLR reaction was performed in the instant application using peripheral blood mononuclear cells (PBMCs), which contain responder T-cells, and allogenic, pre-treated (irradiated) PBMCs, which predominantly contained dendritic cells. As Dr. Fong emphasizes, immunostimulants are important and are very desirable in the treatment of cancer and in enhancing the effectiveness of previously identified treatments for cancer. Supportive evidence also comes from teachings in the art like Steinman *et al.* (Exhibit B) who states that "...**medicine needs therapies that enhance immunity or resistance to infections and tumors.** (page 1, column 1, line 7; emphasis added)". Further teachings like Peterson *et al.* (Exhibit D) show that, recently, the immune stimulant IL-12, was successfully used in a cancer vaccine trial to treat melanoma. Dr. Fong further explains regarding the IL-12 melanoma trial:

> Due to the immune stimulatory effect of IL-12, **the treatment provided superior results** in comparison to earlier work, where the patients' own dendritic cells were prepared from peripheral blood mononuclear cells (PBMCs) treated with antigens, then cultured *in vitro* and returned to the patient to stimulate anti-cancer response" (Emphasis added).

In addition, Dr. Fong's declaration clearly states that:

> A PRO polypeptide shown to stimulate T-cell proliferation in the MLR assay of the present invention with an activity of at least 180% of the control is expected to have the type of activity exhibited by IL-12 and would find practical utility as an immune stimulant.

Accordingly, the positive results obtained in this assay clearly establish the immunostimulant utility for the polypeptides claimed in the present application, and the specification, in turn, enables one skilled in the art to use the compounds for the asserted purpose.

By the foregoing arguments and supportive evidence, Applicants have established that the MLR reaction is a generally recognized assay to assess the immunostimulatory activity.

Regarding "real-life" diseases where the stimulation of lymphocyte proliferation would be beneficial, Applicants submit that in 1998, it was well known in the art, as it is today, that T-cells are highly instrumental in the body's natural defense mechanism fighting infections. For example, viral infections, such as HIV infection, are well known to result in reduced T-cell count. Indeed, the count of T-cell lymphocytes is a generally accepted measure of the extent and seriousness of HIV infection and resultant AIDS. Accordingly, stimulators of T-cell proliferation find utility in fighting viral infections, including retroviral infections, such as HIV infection or Epstein-Barr infection. Furthermore, besides the previously asserted immunostimulatory uses of PRO1343, for example, in the treatment of viral infections like HIV or Epstein Barr viral infections, Applicants assert other utilities in the treatment of cancers like melanoma. Since the legal standard accepts *in vitro* as acceptable utility and the data is "reasonably correlated" to the pharmacological utility based on the discussions above, a valid case for utility has been made and would be considered credible by a person of ordinary skill in the art.

Consequently, polynucleotides encoding PRO1343 meets the requirements of 35 U.S.C. §101 as being useful.

As set forth in M.P.E.P, 2107 II(B)(1), if the applicant has asserted that the claimed invention is useful for any particular practical purpose, and the assertion would be considered credible by a person of ordinary skill in the art, a rejection based on lack of utility should not be imposed. The logic underlying the asserted utilities in the present case is not inconsistent with general knowledge in the art, and would be considered credible by a person skilled in the art. It is, of course, always possible that an invention fails on its way of development to a commercial product. Thus, despite recent advances in rational drug design, a large percentage of drug candidates fails, and never makes it into a drug product. However, the USPTO is not the FDA, the law does not require that a product (drug or diagnostic) be currently available to the public in order to satisfy the utility requirement.

In view of the above, Applicants respectfully submit that PRO1343 has several therapeutic uses, including use in treating join diseases and inflammatory diseases, and that the specification discloses at least one credible, substantial and specific utility for the polypeptide

PRO1343. Accordingly, polynucleotides encoding PRO1343 also meets the requirements of 35 U.S.C. §101 as being useful, hence the Examiner is requested to reconsider and withdraw the rejection of Claims 28-35, 38-40 and 44-47 under 35 U.S.C. §101.

B. Claims 28-47 stand rejected under 35 U.S.C. §112, first paragraph, allegedly for "containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." The Examiner specifically notes that "the deposit of the biological material is considered necessary for the enablement of the current invention."

Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot.

In response, Applicants enclose herewith a copy of the deposit receipt indicating that DNA66675-1587 deposit, ATCC Deposit No. 203282, was made by Applicants on September 22, 1998.

In addition, Applicants respectfully submit that the specification clearly discloses that the deposit was made under the Budapest Treaty and clearly provides the accession number for the deposit, the date of the deposit, the description of the deposited material, and the name and address of the depository starting on page 517, line 1 of the specification.

Applicants further submit that the specification has been amended to recite that the deposit will be maintained "for 30 years from the date of deposit and for at least five (5) years after the most recent request for the furnishing of a sample of the deposit received by the depository" and to recite that "all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of the pertinent U.S. patent."

Accordingly, Applicants believe that the present rejection should be withdrawn.

C. The Examiner further alleges that even if Claims 28-47 possessed utility under 35 U.S.C. §101, which Applicants assert they do, "Claims 28-47 would still be rejected under

35 U.S.C. §112, first paragraph, because the specification, while then being enabling for SEQ ID NO:247 and 248, does not reasonably provide enablement for polynucleotides or polypeptides having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NO:247 or 248, to the protein encoded by ATCC No. 203282, for the extracellular domain thereof, or for vectors and host cells containing these polynucleotides." In addition, the Examiner alleges that "[t]he claims are too broad ... because the claims have no functional limitation."

For all the reasons discussed above, Applicants respectfully disagree with the Examiner. As described above, Applicants respectfully submit that the specification provides sufficient disclosure to establish a specific, substantial and credible utility for the PRO1343 polypeptide and its encoding nucleic acids. In addition, as amended, Claims 28-32 (and, as a consequence, those claims dependent from the same) now recite a functional limitation, namely that the encoded polypeptide induces chondrocyte re-differentiation.

Accordingly, Applicants respectfully submit that it would not require undue experimentation for one of skill in the art to apply the teachings of the present disclosure so as to practice the invention of Claims 28-32 (and, as a consequence, those claims dependent from the same). The Examiner is therefore, respectfully requested to reconsider and withdraw the rejection of these claims under 35 U.S.C. §112, first paragraph.

8. Claim Rejections Under 35 U.S.C. §112, First Paragraph (Written Description)

Claims 28-47 are rejected under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. In particular, the Examiner notes that "[t]he claims are drawn to polynucleotides having at least 80%, 85%, 90%, 95% or 99% sequence identity with SEQ ID NO:247 as well as vectors and host cells[, without requiring] that the polynucleotides or encoded polypeptides of the present invention possess any particular biological activity . . . ."

Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot.

Without acquiescing to the propriety of this rejection, solely in the interest of expediting prosecution in this case, Applicants respectfully submit that amended Claims 28-32 (and, as a consequence, those claims dependent from the same) now recite a functional limitation that the encoded polypeptide induces chondrocyte re-differentiation. Accordingly, it is no longer true that the claims are drawn to a genus of polypeptides defined by sequence identity alone. Coupled with the general knowledge available in the art at the time of the invention, the specification provides ample written support for such polypeptides in Example 150 (page 512 of the specification) where assay for the ability of polypeptides to induce chondrocyte re-differentiation is described. Thus, based on the high percentage of sequence identity and the described method to assay for induction of chondrocyte re-differentiation, one skilled in the art would have known at the time of the invention, that the Applicants had possession of the claimed polypeptides and polynucleotides.

The Examiner is therefore respectfully requested to reconsider and withdraw the rejection of these claims for allegedly lacking written support.

9. Claim Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 28-47 are rejected under 35 U.S.C. §112, second paragraph, for allegedly "being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." The Examiner notes that PRO1343 is "a soluble protein" and is not "disclosed as being expressed on a cell surface. Accordingly, the Examiner states that the limitation that the claimed protein comprises an "'extracellular domain'" and the recitation of "'the extracellular domain ... lacking its associated signal sequence'" is indefinite.

Without acquiescing to the propriety of this rejection, solely in the interest of expediting prosecution in this case, Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot. Further, terms "extracellular domain" or the "extracellular domain ... lacking its associated signal sequence" are no longer present in Claims 28-32 (and, as a consequence, those claims dependent from the same). Accordingly, Applicants request that the rejection of Claims 28-35, 38-40 and 44-47 under 35 U.S.C. §112,

second paragraph, be withdrawn.

10. Claim Rejections Under 35 U.S.C. §102

The examiner noted that the priority of the instant application is set at December 7, 2001. As discussed above, Applicants respectfully submit that the effective filing date of the present application is July 20, 1999 or February 18, 2000 depending on the claimed utility.

Claims 28-47 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Ashkenazi *et al.*, WO 00/53758, publication date of September, 2000. Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot. Claims 28-32 (and, as a consequence, those claims dependent from the same) have been amended to recite a functional limitation that the encoded polypeptide induces chondrocyte re-differentiation.. Accordingly, as discussed above, Applicants are entitled to an effective filing date of February 18, 2000 for subject matters of the instant claims directed to the skin vascular permeability activity and chondrocyte re-differentiation activity. Therefore, as amended, Claims 28-32 (and, as a consequence, those claims dependent from the same) are entitled to an effective filing date of February 18, 2000. Claims 48-52 (and, as a consequence, those claims dependent from the same) are also entitled to an effective filing date of February 18, 2000. Claims 53-57 (and, as a consequence, those claims dependent from the same) are entitled to an effective filing date of July 20, 1999. Hence, Ashkenazi *et al.* is not prior art under 102(b) since its publication date is after the effective priority dates of this application. Accordingly, Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

In conclusion, the present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited. Should there be any further issues outstanding, the Examiner is invited to contact the undersigned attorney at the telephone number shown below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (Attorney's Docket No. **39780-2830 P1C57**).

Respectfully submitted,

Date: September 9, 2004

By: _____
Anna L. Barry (Reg. No. 51,436)

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SV 2053442 v2
9/8/04 4:15 PM (39780.2830)

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Genentech, Inc.
Attn: Ginger R. Dreger
1 DNA Way
South San Francisco, CA 94080-4990

Deposited on Behalf of: Genentech, Inc.

Identification Reference by Depositor:	**ATCC Designation**
pINCY-based plasmid DNA68874-1622 (Ref. PR1622)	203277
pBluescript ®SK-based plasmid DNA64842-1632 (Ref. PR1632)	203278
pSPORT1-based plasmid DNA66660-1585 (Ref. PR1585)	203279
pINCY-based plasmid DNA68871-1638 (Ref. PR1638)	203280
pINCY-based plasmid DNA66674-1599 (Ref. PR1599)	203281
pINCY-based plasmidDNA66675-1587 (Ref. PR1587)	203282

The deposits were accompanied by: ___ a scientific description __ a proposed taxonomic description indicated above. The deposits were received September 22, 1998 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will not inform you of requests for the strains.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested October 2, 1998 On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Barbara M. Hailey, Administrator, Patent Depository

Date: October 7, 1998

The fluorescence change from baseline to the maximum rise of the curve (Δ change) was calculated, and replicates averaged. The rate of fluorescence increase was monitored, and only those samples which had a Δ change greater than 1000 and a rise within 60 seconds, were considered positive.

The following PRO polypeptides tested positive in this assay: PRO1246 and PRO1561.

EXAMPLE 141: Skin Vascular Permeability Assay (Assay 64)

This assay shows that certain polypeptides of the invention stimulate an immune response and induce inflammation by inducing mononuclear cell, eosinophil and PMN infiltration at the site of injection of the animal. Compounds which stimulate an immune response are useful therapeutically where stimulation of an immune response is beneficial. This skin vascular permeability assay is conducted as follows. Hairless guinea pigs weighing 350 grams or more are anesthetized with ketamine (75-80 mg/Kg) and 5 mg/Kg xylazine intramuscularly (IM). A sample of purified polypeptide of the invention or a conditioned media test sample is injected intradermally onto the backs of the test animals with 100 μl per injection site. It is possible to have about 10-30, preferably about 16-24, injection sites per animal. One μl of Evans blue dye (1% in physiologic buffered saline) is injected intracardially. Blemishes at the injection sites are then measured (mm diameter) at 1 hr and 6 hr post injection. Animals were sacrificed at 6 hrs after injection. Each skin injection site is biopsied and fixed in formalin. The skins are then prepared for histopathologic evaluation. Each site is evaluated for inflammatory cell infiltration into the skin. Sites with visible inflammatory cell inflammation are scored as positive. Inflammatory cells may be neutrophilic, eosinophilic, monocytic or lymphocytic. At least a minimal perivascular infiltrate at the injection site is scored as positve, no infiltrate at the site of injection is scored as negative.

The following polypeptide tested positive in this assay: PRO1283, PRO1325 and PRO1343.

EXAMPLE 142: Induction of c-fos in Endothelial Cells (Assay 34)

This assay is designed to determine whether PRO polypeptides show the ability to induce c-fos in endothelial cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of conditions or disorders where angiogenesis would be beneficial including, for example, wound healing, and the like (as would agonists of these PRO polypeptides). Antagonists of the PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of cancerous tumors.

Human venous umbilical vein endothelial cells (HUVEC, Cell Systems) in growth media (50% Ham's F12 w/o GHT: low glucose, and 50% DMEM without glycine: with NaHCO3, 1% glutamine, 10 mM HEPES, 10% FBS, 10 ng/ml bFGF) were plated on 96-well microtiter plates at a cell density of $1x10^4$ cells/well. The day after plating, the cells were starved by removing the growth media and treating the cells with 100 μl/well test samples and controls (positive control = growth media; negative control = Protein 32 buffer = 10 mM HEPES, 140 mM NaCl, 4% (w/v) mannitol, pH 6.8). The cells were incubated for 30 minutes at 37°C, in 5% CO_2. The samples were removed, and the first part of the bDNA kit protocol (Chiron Diagnostics, cat. #6005-037) was followed, where each capitalized reagent/buffer listed below was available from the kit.

> 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of DNA60765-1533 occurred in primary lung tumors LT3, LT12, LT13, LT15, LT16 and LT17. The ΔCt values of these hits are 1.03, 2.17, 2.24, 3.51, 3.32 and 1.02. This represents an increase in gene copy of approximately 2.04, 4.50, 4.72, 11.39, 9.99 and 2.03.

Amplification has also been confirmed framework mapping for DNA60764-1533 in LT16. The reported ΔCt value was 1.37, which represents a 2.58 fold increase in gene copy relative to normal tissue. Epicenter mapping has also confirmed amplification of DNA60764-1533 in LT12, LT13, LT15, LT16, CT1, CT4, CT5, CT7 and CT11. These tumors report ΔCt values of 2.35, 2.37, 3.88, 3.32 in the lung tumors and 1.74, 1.86, 3.28, 1.29 and 2.32 in the colon tumors. Relative to normal tissue, this represents an increase in gene copy of approximately 5.10, 5.17, 14.72 and 9.98 in the lung tumors and 3.34, 3.63, 9.71, 2.45 and 4.99 in the colon tumors.

In contrast, the amplification of the closest known framework markers, epicenter markers and the comparison sequences does not occur to a greater extent than that of DNA60764-1533. This strongly suggests that DNA60764-1533 is the gene responsible for the amplification of the particular region in Chromosome 19. Because amplification of DNA60764-1533 occurs in various lung and colon tumors, it is highly probably to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA60764-1533 would be expected to have utility in cancer therapy.

PRO1317 (DNA71166-1685):

The ΔCt values for DNA71166-1685 in a variety of tumors are reported above. A ΔCt of > 1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. The above data indicates that significant amplification of nucleic acid DNA71166-1685 encoding PRO1317 occurred in primary lung tumors LT1, LT1a, LT9, LT10, LT15, LT17 and LT22 . Because amplification of DNA71166-1685 occurs in various tumors, it is likely associated with tumor formation and/or growth. As a result, antagonists (*e.g.*, antibodies) directed against PRO1317 would be expected to be useful in cancer therapy.

Summary

Because amplification of the various DNA's as described above occurs in various tumors, they are likely associated with tumor formation and/or growth. As a result, antagonists (*e.g.*, antibodies) directed against these polypeptides would be expected to be useful in cancer therapy.

EXAMPLE 144: Stimulatory Activity in Mixed Lymphocyte Reaction (MLR) Assay (Assay 24)

This example shows that certain polypeptides of the invention are active as a stimulator of the proliferation of stimulated T-lymphocytes. Compounds which stimulate proliferation of lymphocytes are useful therapeutically where enhancement of an immune response is beneficial. A therapeutic agent may take the form of antagonists of the polypeptide of the invention, for example, murine-human chimeric, humanized or human antibodies against the polypeptide.

The basic protocol for this assay is described in Current Protocols in Immunology, unit 3.12; edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Insitutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO_2) and then washed and resuspended to $3x10^6$ cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate). The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads).

The assay is prepared by plating in triplicate wells a mixture of:

100:l of test sample diluted to 1% or to 0.1%,

50 :l of irradiated stimulator cells, and

50 :l of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO_2 for 4 days. On day 5, each well is pulsed with tritiated thymidine (1.0 mC/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to $1x10^7$ cells/ml of assay media. The assay is then conducted as described above.

Positive increases over control are considered positive with increases of greater than or equal to 180% being preferred. However, any value greater than control indicates a stimulatory effect for the test protein.

The following PRO polypeptides tested positive in this assay: PRO1246 and PRO1343.

EXAMPLE 145: Mouse Kidney Mesangial Cell Proliferation Assay (Assay 92)

This assay shows that certain polypeptides of the invention act to induce proliferation of mammalian kidney mesangial cells and, therefore, are useful for treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with Schönlein-Henoch purpura, celiac disease, dermatitis herpetiformis or Crohn disease. The assay is performed as follows. On day one, mouse kidney mesangial cells are plated on a 96 well plate in growth media (3:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium, 95% fetal bovine serum, 5% supplemented with 14 mM HEPES) and grown overnight. On day 2, PRO polypeptides are diluted at 2 concentrations(1% and 0.1%) in serum-free medium and added to the cells. Control samples are serum-free medium alone. On day 4, 20μl of the Cell Titer 96 Aqueous one solution reagent (Progema) was added to each well and the colormetric reaction was allowed to proceed for 2 hours. The absorbance (OD) is then measured at 490 nm. A positive in the assay is anything that gives an absorbance reading which is at least 15% above the control reading.

EXAMPLE 149: Detection of PRO Polypeptides That Affect Glucose or FFA Uptake by Primary Rat Adipocytes (Assay 94)

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by adipocyte cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by adipocytes would be beneficial including, for example, obesity, diabetes or hyper- or hypo-insulinemia.

In a 96 well format, PRO polypeptides to be assayed are added to primary rat adipocytes, and allowed to incubate overnight. Samples are taken at 4 and 16 hours and assayed for glycerol, glucose and FFA uptake. After the 16 hour incubation, insulin is added to the media and allowed to incubate for 4 hours. At this time, a sample is taken and glycerol, glucose and FFA uptake is measured. Media containing insulin without the PRO polypeptide is used as a positive reference control. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as stimulators of glucose and/or FFA uptake in this assay: PRO1265, PRO1283, PRO1279, PRO1303, PRO1306, PRO1325, PRO1565 and PRO1567.

The following PRO polypeptides tested positive as inhibitors of glucose and/or FFA uptake in this assay: PRO1194, PRO1190, PRO1326, PRO1343, PRO1480, PRO1474, PRO1575 and PRO1760.

EXAMPLE 150: Chondrocyte Re-differentiation Assay (Assay 110)

This assay shows that certain polypeptides of the invention act to induce redifferentiation of chondrocytes, therefore, are expected to be useful for the treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis. The assay is performed as follows. Porcine chondrocytes are isolated by overnight collagenase digestion of articulary cartilage of metacarpophalangeal joints of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm^2 in Ham F-12 containing 10% FBS and 4 μg/ml gentamycin. The culture media is changed every third day and the cells are then seeded in 96 well plates at 5,000 cells/well in 100μl of the same media without serum and 100 μl of the test PRO polypeptide, 5 nM staurosporin (positive control) or medium alone (negative control) is added to give a final volume of 200 μl/well. After 5 days of incubation at 37°C, a picture of each well is taken and the differentiation state of the chondrocytes is determined. A positive result in the assay occurs when the redifferentiation of the chondrocytes is determined to be more similar to the positive control than the negative control.

The following polypeptide tested positive in this assay: PRO1265, PRO1250, PRO1430, PRO1356, PRO1275, PRO1274, PRO1286, PRO1273, PRO1283, PRO1279, PRO1306, PRO1325, PRO1343, PRO1418, PRO1565, PRO1474, PRO1787, PRO1556 and PRO1801.

EXAMPLE 151: Induction of Pancreatic β-Cell Precursor Proliferation (Assay 117)

This assay shows that certain polypeptides of the invention act to induce an increase in the number of pancreatic β-cell precursor cells and, therefore, are useful for treating various insulin deficient states in mammals, including diabetes mellitus. The assay is performed as follows. The assay uses a primary culture of mouse fetal pancreatic cells and the primary readout is an alteration in the expression of markers that represent

EXAMPLE 2

Stimulatory Activity in Mixed Lymphocyte Reaction (MLR) Assay (no.24)

This example shows that the polypeptides of the invention are active as a stimulator of the proliferation of stimulated T-lymphocytes. Compounds which stimulate proliferation of lymphocytes are useful therapeutically where enhancement of an immune response is beneficial. A therapeutic agent may take the form of antagonists of the polypeptide of the invention, for example, murine-human chimeric, humanized or human antibodies against the polypeptide.

The basic protocol for this assay is described in Current Protocols in Immunology, unit 3.12; edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Insitutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37 ^{0}C, 5% CO_2)and then washed and resuspended to 3 x 10^6 cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate).

The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads). The assay is prepared by plating in triplicate wells a mixture of:

100:l of test sample diluted to 1% or to 0.1%

50 :l of irradiated stimulator cells and

50 :l of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37 ^{0}C, 5% CO_2 for 4 days. On day 5 and each well is pulsed with tritiated thymidine (i.0 mC/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI;10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging

at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to 1x 10^7 cells/ml of assay media. The assay is then conducted as described above. The results of this assay for compounds of the invention are shown below. Positive increases over control are considered positive with increases of greater than or equal to 180% being preferred. However, any value greater than control indicates a stimulatory effect for the test protein.

Table

PRO	PRO Concentration	Percent Increase Over Control
PRO256	1.80nM	261.0
"	0.225nM	202.0
"	0.028nM	150.0
PRO306	0.55nM	204.1
"	5.46nM	267.7
PRO364	2.72nM	120.2
"	27.23nM	278.4
PRO826	0.9nM	144.8
"	0.9nM	249.4
"	9.03nM	191.9
"	9.03nM	213.6
PRO1068	1.67nM	121.0
"	1.67nM	229.9
"	16.72nM	224.8
"	16.72nM	276.3
PRO1343	2.3nM	205.1
"	2.3nM	245.6
"	230nM	107.7
"	230nM	122.0
PRO1375	5.9nM	103.1
"	5.9nM	141.2
"	59nM	212.5
"	59nM	221.7

EXAMPLE 3

Skin Vascular Permeability Assay (no.64)

This assay shows that certain polypeptides of the invention stimulate an immune response and induce inflammation by inducing mononuclear cell, eosinophil and PMN infiltration at the site of injection of the animal. This skin vascular permeability assay is conducted as follows. Hairless guinea pigs weighing 350 grams or more are anesthetized with ketamine (75-80 mg/Kg) and 5 mg/Kg xylazine